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Neurotransmitters and Ischemic Neurological Dysfunction in Gerbils

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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Neurotransmitters and Ischemic Neurological Dysfunction in Gerbils

Master of Science

1984

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Neurotransmitters and Ischemic Neurological Dysfunction in Gerbils submitted by Masako Walley in partial fulfilment of the requirements for the degree of Master of Science in Experimental Medicine.

T. Nilsei

Supervisor

DATE November 21, 1983

DEDICATION

This thesis is dedicated to Roc who is my best friend and also my husband who has shared all the difficulties I had to cope with.

ABSTRACT

The effect of ischemia on the neurotransmitters, norepinephrine (NE), dopamine (DA), and γ-aminobutyric acid (GABA), was studied in the gerbil stroke model. After unilateral carotid occlusion, the animals were separated into groups according to the observed neurological effects; 1) neurological deficit 2) seizures, and 3) asymptomatic. Animals with neurological deficit were given Naloxone or placebo (saline); those with seizures were given clonidine or placebo. Measurements were also performed in a control group of sham-operated animals. All animals were sacrificed 3 hours after carotid occlusion and the brains removed for neurotransmitter assay.

Untreated gerbils with ischemia-induced seizures showed highly significant increases in NE and GABA, and a decrease in DA in the ischemic hemisphere. Clonidine treatment abolished the seizures and prevented the changes in NE and DA but did not affect the increase in GABA. Neurological deficits such as paralysis were unaffected by naloxone: In this group, untreated animals had significant changes in GABA only while naloxone-treated animals had a decrease in DA in the ischemic hemisphere. In gerbils which appeared normal after carotid occlusion, morphine generally caused neurological deficits to appear; these animals demonstrated significant changes in DA (decrease) in the ischemic hemisphere. Untreated normal animals had no changes in NE and DA, but GABA was increased.

The results of this study do not support a benefical effect of naloxone in focal cerebral ischemia. They do, however, provide evidence that use of the gerbil stroke model requires the treatment of animals with post-ischemic seizures as a separate group. Marked alterations in

neurotransmitter levels occurred during ischemia and the type of degree of the neurotransmitter changes were related to the severity of neurological dysfunction. Clonidine abortion of ischemic seizures with inhibition of NE release may be of clinical significance and warrants further study.

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CHAPTER I

INTRODUCTION

The term stroke is commonly used to denote a sudden onset of neurological dysfunction due to acute impairment of cerebral blood flow (CBF). In approximately 70% of cases, the primary event consists of a cerebral arterial occlusion by a local thrombus formation or embolus. Subsequently, a variable amount of brain tissue will suffer ischemic damage, the extent of damage being primarily determined by factors such as the availability of collateral blood supply and the duration of ischemia.

Despite extensive research, the changes in cerebral tissue function and metabolism which occur during the initial few minutes and hours after onset of ischemia are not well understood. Although the relationship between the severity of ischemia, impairment of neurological function, and failure of energy metabolism have recently been determined, there is little knowledge about the factors which result in progression from simple reversible functional impairment of neurons to irreversible damage causing cell death. It has recently been suggested that disorders of neurotransmitters observed in cerebral ischemia may be responsible for the progression of post-ischemic brain damage.

A lack of understanding of the factors which limit cerebral tissue survival during acute ischemia is probably the major reason why efforts to treat stroke patients during the first few hours have been few and unsuccessful. Some investigators, however, using animal models of focal ischemia, have recently reported dramatic improvement in survival and

neurological dysfunction following treatment with opiate anagonists.

The present study was undertaken with a view to further assessing the effectiveness of opiate antagonism and its mechanism in the treatment of acute focal ischemia. The hypotheses tested were: 1) that disturbances in brain neurotransmitters are related to the presence and type of ischemic neurological dysfunction, and 2) that opiate antagonists exert their therapeutic influence via neurotransmitters.

In Chapter II, a review of the current knowledge of the pathophysiology of focal cerebral ischemia is presented. Particular emphasis is given to the studies which have been concerned with ischemia-induced alteration in brain neurotransmitters.

In Chapter III, a review of recent evidence for the effectiveness of opiate antagonists in the treatment of focal ischemia is presented. Chapters IV, V and VI are concerned with the methodology results and discussion of the present study.

CHAPTER II

PATHOPHYSIOLOGY OF FOCAL ISCHEMIA

A. Ischemic Threshold

Interruption of cerebral blood flow results in loss of consciousness and cessation of spontaneous and evoked electrical activity (Astrup et al., 1981). With loss of electrical activity there may also be a major disruption of ion homeostasis.

Recent evidence indicates that disturbance of brain function is critically dependent on residual blood flow. The development of infarction is similarly correlated to residual perfusion, and there is a lethal threshold of residual blood flow below which tissue infarction develops after a certain time.

Normal regional cerebral blood flow in animals and man is about 0.55 ml/gm/min. Levy et al., (1979) and Symon et al., (1974) demonstrated that electrical function in the baboon's cortex was abolished at local flows below about 0.2 ml/gm/min., but was sustained above this level. Therefore, this has been referred to as the flow threshold of electrical failure in the cerebral cortex. Since oxygen uptake at the electrical threshold supposedly was somewhat reduced, energy failure with efflux of cellular potassium and membrane depolarization was suspected as the cause of electrical failure. However, Astrup and his colleagues (1977) did not find increased extracellular potassium concentration, indicative of "pump failure," at the threshold when electrical function ceased.

In a subsequent study, Branston (1977) determined a critical ischemic threshold of about 0.10 ml/gm/min. below which the

extracellular potassium concentration increased massively due to efflux of potassium from the cell. Symon and Brierly (1976) found that in chronic ischemic infarction, the area in which infarction developed corresponded to the zones which had flow rates of less than 0.1 ml/gm/min., immediately following acute occlusion. Morawet et al., (1978) also found that recovery without histological signs of infarction could only be found at local blood flow about 0.12 ml/gm/min. following a 2 to 3 hour period of focal ischemia in the monkey.

The concept of a flow threshold for infarction and its possible relation to the threshold for ion pump failure needs to be further investigated. It is clear that energy state and ion homeostasis are not the factor per se indicating irreversible damage since they both can be fully recovered even after prolonged periods of normothermic ischemia (Ljunggren et al., 1974; Hossman et al., 1977) from which recovery of integrated cerebral functions has not yet been possible. Electrical activity, energy metabolism and ion pumping respond immediately to appropriately reduced supply of oxygen while the development of infarction appears to have a considerable time factor involved (Astrup et al., 1981).

B. Ionic Changes During Ischemia

In addition to the rise in extracellular fluid concentration of potassium, severe ischemia results in other ionic fluxes.

With the advent of potassium sensitive microelectrodes it could be shown that upon interruption of cerebral circulation, a massive efflux of potassium occurs within 1-2 min (Hossman et al., 1977; Astrup et al., 1980). At the same time a large transfer of sodium from the extracellular to the intracellular space occurs.

With calcium sensitive electrodes, ischemia was also shown to be associated with a marked decrease in extracellular calcium activity (Nicholson, 1980; Harrison et al., 1981). First, intracellular calcium rises because sodium as well as the free fatty acids stimulate the release of calcium from the mitochondria (tissue free fatty acid levels rise during ischemia), and second, calcium is released from the endoplasmic reticulum because of an ischemia-induced shortage of adenosine triphosphate (ATP) (Rehncrona et al., 1982). Calcium-induced loss of membrane phospholipids probably alters the equilibrium between the membrane protein lipid such that membrane calcium permeability is drastically altered (Farber et al., 1981). Both depletion of phospholipids and metabolism of free fatty acids have been implicated with irreversible damage to ischemic cells.

C. Free Fatty Acids and its Metabolites

Free fatty acids, particularly the metabolites of arachidonic acid, have a variety of detrimental effects on brain structure and function.

Arachidonic acid can initiate an important cascade of biochemical events leading to the production of the eicosanoids (i.e. prostaglandins, thromboxanes and leukotrienes) (Wolfe, 1982). In the absence of even low levels of oxygen in the tissue (i.e. incomplete ischemia), the formation of prostaglandins, thromboxanes, and leukatrienes may proceed. Each of these groups of compounds contains individual members with the potential for cell damage.

i) Prostaglandins

Several authors have demonstrated that prostaglandins are present in brain vessels, especially prostacyclin (PGI_2) which is synthesized in the endothelial cells and which is a vasodilator and inhibitor of

platelet aggregation (Marshall et al., 1975: Moncada and Vane Jr., 1979). The prostaglandins have been implicated in various cerebrovascular disorders connected with brain hypoxia, ischemia, etc. (Gaudet and Levine, 1980). Raichle (1983) speculated that the biosynthesis of the PGI_2 is augmented in the partially ischemic or reperfused brain because of the increased concent ration of arachidonic acid. However, the positive effects of PGI_2 might be prevented by an accumulation of by-products during the biosynthesis.

ii) Thromboxane and Leukotriene

In addition to the synthesis of prostaglandin, arachidonic acid serves as the precursor of another important group of compounds known as thromboxane and leukotriene.

Thromboxane is formed primarily from platelets in the microvasculature (Wolfe, 1982). The major actions of thromboxane are to promote platelet aggregation and induce vasoconstriction. Thus, thromboxane directly opposes the actions of prostacyclin (Wolfe, 1982).

Leukotriene contract smooth muscle and alter the permeability of cell membranes. There is already preliminary evidence that at least some of these compounds are synthesized in the mammalian brain (Wolfe, 1982). Raichle speculated that the leukotrienes could well contribute to the increased flux of calcium into the injured cell in addition to altered cell membrane permeability (1982).

D. Perturbation of Cellular Metabolism

Energy depletion, defined in terms of the tissue concentrations of phosphocreatine, ATP, adenosine diphosphate, and adenosine monophosphate occurs in ischemic brain tissue. Levy and Duffy (1977) reported that the residual perfusion of blood supply may provide sufficient oxygen to

maintain a close to normal tissue concentration of ATP. However, the concentration of phosphocreatinine and lactate are greatly reduced and increased respectively. Since the concentrations of adenosine diphosphate and adenosine monophosphate are moderately increased, Astrup et al., (1981) suggested some degree of energy failure during ischemia. Dalford and his colleagues (1973) reported in their hypoxia study that such moderate energy imbalance does not lead to neuronal damage.

Ischemia is invariably accompanied by an increase in the tissue lactate concentration and a fall in tissue pH due to anaerobic metabolism. Myers (1979) reported the effect of pre-ischemic nutritional state in the outcome following prolonged periods of ischemia or hypoxia: fasted animals had a better outcome than animals with high blood glucose level. He attributed this difference in outcome to the difference in tissue lactate levels in the two groups. The fasted groups of animals had significantly lower lactate levels than the other group of animals with a high blood glucose level. Rehncrona et al., (1980) similarly found that a negative correlation between the degree of ischemia-induced tissue lactacidosis (varied by altering the pre-ischemic nutrition state of the animal) and the degree to which the cerebral energy state is restored toward normal following ischemia. Animals with tissue lactate levels in excess of 20 to 25 mmol per kilogram of body weight failed to recover a "normal" energy state.

E. Neurotransmitters

Disturbances in cellular metabolism during ischemia may affect the metabolism of brain neurotransmitters. Recently some investigators have suggested that altered levels of cerebral neurotransmitters may play an important role in the pathogenesis of cerebral ischemia (Osterholar and

Mathew, 1972). The following section will review the changes in norepinephrine (NE), dopamine (DA), 5-hydroxytryptamine. (5HT; Serotonin) and -aminotutyric acid (GABA) during cerebral ischemia.

1) Norepinephrine

Meyer et al., (1973) reported catecholamine concentrations in cerebrospinal fluid and plasma of patients with cerebral infarction. In patients with cerebral infarction of less than two weeks duration, the total concentration of catecholamines (NE, epinephrine) in both CSF and plasma was significantly elevated when compared to the small group of patients without stroke. Since Toyoda and Meyer (1969) pointed out that the most potent stimulus for the release of epinephrine was a rapid and sharp decrease in cerebral blood flow, they suggested that the NE release might be triggered by the blood pressure change and stress associated with the onset of cerebral ischemia (Meyer et al., 1973). Zervas et al., (1974) anesthetized mature mongolian gerbils (60-70 g) with diethyl ether and ligated the left common carotid artery. Brain NE concentrations were not dissimilar ipsilateral and contralateral to the lesion after 24 h. They attributed the lack of change in NE after carotid ligation to the following mechanisms:

1. The caudal location in the brain stem of NE bodies which would continue to be nourished by the Basilar artery;
2. The existence in gerbils of a single anterior cerebral artery which would allow NE terminals in the anterior pole of the left hemisphere to be nourished by blood from the right common corotid artery; and
3. Less susceptability of NE neurons to anoxia.

However, Lust et al., (1975) recorded a quite different NE level in

their experiments. They investigated the changes in putative neurotransmitters and cyclic nucleotides and found that the level of NE decrease was evident after 30 minutes and became significant by 6 hours (22% of control valves). They also measured ATP and phosphocreatine to provide biochemical evidence for ischemia. Both ATP and phosphocreatine levels in the ischemic cerebral cortex decreased to less than 25% of control valve by 1 hour and remained depressed for up to 6 hours of ligation. Interestingly, in a study of catecholamine content of cerebral tissue after occlusion of middle cerebral artery (MCA) in cats, great variability of NE-epinephrine (E) content was reported (Cohen et al., 1975). The unilateral operation appeared to have a transient effect on the NE-E content of bilateral cerebral tissue. Values were low at 16 and 24 minutes and high for three of four samples after 4 hours after the surgical procedure. There were no consistent side to side differences of NE-E content after MCA occlusion, although at 6 hours, 24 hours, and 7 days values for the side of occlusion were lower than those for the opposite side.

Lavyne et al., (1975) investigated the effect of carotid ligation on brain catecholamine metabolism in gerbils by administering [^{3}H] DA intraventricularly prior to performing the ligation, and measuring brain [^{3}H]-metabolites 24 hours later. NE was significantly reduced ipsilateral to the ligation. The reduction in endogenous NE ipsilateral to the lesion was associated with greater decrease in brain [^{3}H] NE concentration. They hypothesized that the reduction was not just due to impaired NE synthesis, but also increased release from the NE neurons.

Kogure et al., (1975) hypothesized that ischemia produces a generalized release of NE from presynaptic terminals throughout the

brain, constricting cerebral vessels causing wide spread ischemia, even in areas distant from the site of occlusion. They produced local ischemia in rats by internal carotid artery injection of 34 carbon microspheres and compared the NE and cyclic adenosine 3', 5' - monophosphate (cAMP) level in embolized and intact hemispheres. Tissue concentration of cAMP and NE were measured serially at 0, 2, 5, 30, and 60 minutes and 4 hours. There was an instantaneous increase of CAMP in the embolized hemisphere and more gradual increase in the non-embolized hemisphere. NE was reduced bilaterally by 2 minutes following completion of the injection of the microspheres, and thereafter, returned gradually toward the control levels, but was still low 4 hours after embolization.

The behaviour of biogenic amines during unilateral ischemia was studied in the brains of mongolian gerbils by Mrsulja et al., (1976). Compared to baseline measurements, there were no differences in content of NE between two hemispheres after 15 minutes of carotid ligation; however, the NE level in the ischemic hemisphere at 1 hour after onset of ischemia was reduced and at 3 hours the change was more pronounced. The behavior of NE during reperfusion (clip released) differed in gerbils occluded for shorter periods (15 minutes and 1 hour) as compared to those subjected to ischemia of longer duration (3 and 6 hours). In the short period group the post-ischemic NE levels showed a biphasic course with significant elevations reached 1 hour following release of clip, and followed by depressions below control levels. The NE levels of longer duration group showed gradual recovery after initial reduction during the ischemic periods. Following 3 hours of occlusion, the NE levels rose within 1 hour from the lowest point of depression at the time of clip release to values which were not significantly different

from the controls. The return of NE to normal levels in 6 hour occluded gerbils was evident at 20 hours after release. Mrsulja et al., (1976) described the above results as an example of the "Maturation" phenomenon after an ischemic lesion.

Similarly, Gaudet et al., (1976) investigated the effect of transient ischemia on monoamine levels in the cerebral cortex of gerbils. The NE level was significantly reduced in the occluded hemispheres after 60 minutes, whereas values remained unaltered in the non-stroke group. The NE level during cerebral reperfusion for up to 30 minutes after the prior 60 minutes of arterial occlusion caused a bilateral increase in the NE level above control values in both stroked and non-stroked animals except in the occluded hemispheres of the former. The NE increased to almost twice the control value, in both stroked and non-stroked animals 15 and 30 minutes after reperfusion except in occluded hemisphere. However, 60 minutes after reperfusion, NE levels fell below control in the occluded hemisphere of both animal groups. The authors speculated that the "catecholamine rebound" is related to reactive hyperemia or hyperoxia in brain since tissue oxygen tension and CBF returned to steady state value after 30 minutes of cortical reperfusion at which time catecholamine rebound was at its peak. Most of the stroked animals showed a return of circling behavior and in some animals seizure activity appeared at a time which approximately coincided with that of the catecholamine rebound. Interestingly, Mason and Corcoran (1979) reported that a significant potentiation of the seizure induced both by Metrazol and by electroconvulsive shock was found in animals depleted of NE.

Mrsulja and his colleagues (1976) also investigated the changes in

NE level in gerbil brain ischemia and effects of alpha-methyl-P-tyrosine (AMPT) and pargyline. Unilateral ligation of corotid artery for two hours produced significant reduction of NE level in the ipsilateral hemisphere. No changes were found in the contralateral hemisphere nor in sham-operated animals. Additional NE reduction was observed in animals treated with AMPT prior to the ischemic exposure. Pretreatment with pargyline produced lower accumulation of NE in ischemic animals. Relative uptake of [^{3}H] NE was increased about 62% in a synaptosome preparation obtained from the ischemic brain. They concluded that their results support the contention of increased NE release occurring in ischemia.

Harrison et al., (1979) investigated the effect of gerbils' unilateral carotid ligation on brain neurotransmitters after 3.5 hours. There appeared to be a trend toward lower NE levels on the ischemic side in both clinically affected and unaffected groups. No obvious differences were seen in the MOPEG sulfate level (4 hydroxy-3-methoxy phenylethylene glycol sulfate). They felt that there was a relationship between their NE findings and the nature of the neurological abnormality produced during ischemia (seizure vs. paralysis). Since they did not separate their neurologically affected animals, they suggested that investigators separate their animals into subgroups in future studies.

Pau et al., (1982) used adult rabbits and ligated the middle cerebral artery to observe the changes in NE levels. NE levels significantly ($P<0.01$) declined 10 minutes after the MCA occlusion (64.3% of the control values), but the reduction was less pronounced after 60 minutes (69.6% of the control values) and not significant after 360 minutes (91.1% of the control values).

Thus, although there is considerable variation in the results obtained, most experimental studies have provided evidence for a significant decrease in NE levels during the first few hours after onset of ischemia. The mechanism responsible for the NE decrease appears to be increased neuronal release with subsequent washout into the CSF and plasma.

ii) Dopamine

Hudgins et al., (1970) subjected 6 squirrel monkeys to transorbital ligation of the left middle cerebral artery, and measured to the DA level three hours after ligation. Ipsilateral to the vascular lesion, the brain DA concentration was significantly lower than the contralateral side ($P<0.02$). Zervas et al., (1974) used mature mongolian gerbils and ligated the left common corotid artery. Twenty-four hours later, brain DA was reduced by 46% on the infarcted side ($P<0.02$). Brain DA was not significantly reduced among gerbils killed two hours after ligation, but was depressed among animals killed after three hours. The largest decreases were in the hypothalamus and nucleus accumbens-olfactory tubercle. Among sham-operated animals in which the common carotid artery was exposed, but not ligated, brain DA was unchanged in all regions examined. Similarly, Lust et al., (1975) found that DA level in the ischemic hemisphere was decreased to 30% of the control hemisphere by 6 hours post-ligation of the left common carotid artery in gerbils.

To obtain additional information about the effect of carotid ligation on brain catecholamine metabolism Lavyne et al., (1975) have administered [^{3}H] DA intraventricularly 4 hours prior to the ligation, and measured brain [^{3}H] metabolites 24 hours later. Ipsilateral

hemispheres of those animals with neurological symptoms contained less than half as much DA as the contralateral hemispheres ($P<0.001$). The reductions in the endogenous DA ipsilateral to the lesion was associated with even greater decreased in brain [^{3}H] DA.

Kogure et al., (1975) produced cerebral infarction by internal carotid injection of 35 carbon microspheres in rats. The effect of microsphere embolism on the cortical concentration of DA was a bilateral increase in DA at 5 minutes. By 4 hours DA returned to normal. The authors speculated that the substantial increase in DA level 5 minutes after embolization was due to a defect in conversion of DA to NE. The authors noted that the time course of the DA change parallels that of the fall and recovery of ATP.

Mrsulja et al., (1976) investigated the changes in DA levels in gerbils subjected to carotid occlusion. In animals occluded for 15 minutes, there were no differences in content of DA between left (ischemic) and right (control) cerebral hemispheres. During the baseline measurement, DA concentration was only slightly reduced at one hour after the left common carotid was occluded, but the reduction was pronounced after 3 and 6 hours of ischemia. In other studies Mrsulja et al., (1976, 1977) reported a significant reduction of DA ($P<0.01$) 2 hours after carotid ligation in gerbils. No changes were found in the contralateral hemisphere nor in sham-operated animals.

Gaudet et al., (1977) also reported the effect of transient ischemia on DA levels in the cerebral cortex of gerbils but found that the DA level did not change during 60 minutes of carotid occlusion either <u>stroked</u> or <u>non-stroked</u> animals. Harrison et al., (1979) also found no changes in cortical DA level 3.5 hours post carotid ligation in

gerbils.

Jellinger and his colleagues (1978) examined brain samples of 16 patients with cerebral infarcts due to thrombotic occlusion of large brain arteries or other circulation disorders. They separated their patients into recent infarcts (4, 6 and 8 hours before death) and older infarcts (5 days to 4 months). In recent infarcts a complete depletion of DA within the necrotic cortex, corpus striatum and white matter were observed. However, the peripheal area showed an almost normal DA level.

Akiguchi et al., (1980) studied the role of central aminergic fibers in experimental cerebral ischemia in stroke-prone spontaneously hypertensive rats (SHRSP). DA fluorescence in the nucleus candidate and putamen and cerebral cortex was marked by depleted along with CBF reduction in symptomatic SHRSP with bilateral corotid artery ligation. Ahagon et al., (1980) investigated alterations of brain DA in the acute stage of cerebral ischemia after a unilateral common corotid ligation in gerbils. The distribution of dopaminergic terminals and cell bodies in gerbils is the same as in other mammals. On the ligated side after one hour of ischemia, diffuse green fluorescence of dopaminergic terminals showed only a slight decrease in intensity when compared to non-ligated side. But white matter and bundles of myelinated fibers adjacent to and in the DA rich regions had an intense green fluorescence in contrast to the non-ligated side where they are normally non-fluorescent. This was considered to indicate the extraneural leakage and diffusion of DA. The intensity of extraneural green fluorescence was especially high in glia cells. There was also an unusual green fluorescence in the lumen of small vessel in DA rich regions on the ligated side, Dopaminergic cell bodies in the substantia nigra on the ligated side revealed a

conspicuous reduction in the fluorescence intensity in severely affected cases. After 2 or 3 hours of ischemia, there was a marked reduction or disappearance of the diffuse green fluorescence on the ligated side. Ahagon et al., (1980) attributed this in part to further diffusion of the leaked DA.

Weinberger and Cohen (1982) investigated the differential effects of ischemia on the active uptake of DA by brain synaptosomes. There was no change in uptake of [^{3}H] DA in synaptosomes obtained from the ischemic hemisphere up to 8 hours post left carotid ligation of gerbils. At 16 hours after ligation, there were marked decrements in uptake of [^{3}H] DA in animals showing hemiparesis. Separate experiments performed with synaptosomes isolated from the corpus striatum showed that sensitivity to damage was intrinsic to the DA nerve terminal and not the result of regional variations in the ischemic damage in brain. No bilateral effect of ischemia on DA uptake was evident. In animals exhibiting milder behavioral deficits (circling), there was a smaller and comparable decrement in uptake of DA. The authors speculated that it is likely that the DA nerve terminal has a greater inherent susceptibility to the factors causing irreversible ischemic neuronal damage. It is evident that the effects of ischemia on DA and NE levels in the brain are very similar. DA is also reduced within a few hours of the onset of ischemia. It appears that increased neuronal release, probably coupled with impaired synthesis and uptake are the mechanisms responsible.

iii) Serotonin

Meyer et al., (1974) measured hemispheric blood flow (HBF) and the levels of monoamines and their metabolites in CSF in 32 patients with

subacute cerebral infarction. Although there was no clear definition of the subacute stage of cerebral infarction, CSF concentrations of 5-HT were high at an early stage after the onset of cerebral infarction and gradually declined as patients showed recovery from neurological deficit during the ensuing two to three weeks. A significant inverse correlation was noted between CSF 5-HT levels and duration of the stroke. There was also a significant inverse correlation between CSF 5-HT concentrations and HBF, i.e. the higher the 5-HT level, the more marked the HBF reduction. Jellinger et al., (1978) analysed brain samples of 16 patients with cerebral infarction due to thrombotic occlusion of large brain arteries. They separated their brain samples into two groups; 10 recent infarcts (4 to 8 hours before death) and 6 old infarcts (5 days to 6 months survival). In recent infarcts a complete depletion within the necrotic cortex corpus striatum and white matter of 5-HT was associated with significant reduction of 5-hydroxyindoleacetic acid (5-HIAA), particularly in the necrotic cortex, to 36% of control values. Edematous brain showed significant accumulation of both 5-HT and 5-HIAA, the elevation of these compounds being more pronounced in the edematous white matter. A similar increase of tryptophan (TRP), the precursor of 5-HT, was seen in both the necrotic zone and perifocal edema of recent infarcts. These findings in human brain infarction indicate that the disorder of cerebral 5-HT metabolism is contributing to the development of post-ischemic brain damage and the complicating cerebral edema.

Harrison et al., (1979) investigated the effect of experimental ischemia on 5-HT in the gerbil brain. Levels of 5-HT and its metabolite 5-HIAA were reduced on the operated side compared with sham-operated

animals, in both clinically affected and unaffected animals by 3.5 hours post unilateral carotid ligation. Both 5-HT and 5-HIAA levels were lower on the unoperated side when compared with sham-operated animals, but not significantly so. The extent of reduction in 5-HT and 5-HIAA on both the operated and unoperated sides was the same, irrespective of whether animals developed neurological deficit or not. There was no significant difference between the two hemispheres in sham-operated animals either for 5-HT or 5-HIAA. Since the reduction of 5-HT was accompanied by a corresponding fall in the level of its metabolites 5-HIAA, 5-HT synthesis rather than metabolism appeared to be inhibited. The authors also speculated that the bilateral drop in cerebral 5-HT and 5-HIAA could be due to damage midbrain raphe nuclei which are the major source of cerebral 5-HT neuron projections.

Welch et al., (1977) reported the influence of p-cholorophenylalanine (PCPA) on 5-HT levels in ischemic gerbils. There was a significant decrease of 5-HT in occluded hemispheres of symptomatic animals and this decrease was significant compared with values in the contralateral non-occluded hemisphere. In 182 PCPA-pretreated animals the stroke incidence was significantly reduced compared with that in untreated animals. Treated animals also appeared to survive longer (up to 48 hours before death), whereas untreated animals usually died within twelve hours of carotid ligation. In symptomatic animals sacrificed between 3 and 48 hours, hemispheric 5-HT levels were bilaterally reduced compared with sham-operated controls and decreased in comparison with asymptomatic animals also sacrificed up to 48 hours after occlusion. The degree of decrease was approximately twice that observed in untreated animals. Reduction of 5-HT in ischemic

brain appeared to be due to synthesis impairment since inhibition of tryptophan hydroxylase activity prior to ischemia resulted in even greater 5-HT depletion than that seen in untreated animals. Bilateral decreases in symptomatic and asymptomatic animals also indicated some 5-HT synthesis impairment even in brain areas remote from the ischemic area. Similarly Mrsulja et al., (1977) reported the effect of PCPA and other drugs known to affect monoamine turnover. The exposure of the gerbils pre-treated with PCPA to ischemia produced additional reduction of 5-HT levels while decrease of 5-HIAA was blocked.

A recent study by Pau et al., (1982) provides additional pertinent information on the effect of ischemia on 5-HT. Occlusion of the MCA in rabbits resulted in a decrease of 5-HT with an associated elevation of 5-HIAA within the brain cortex supplied by the artery. The 5-HT severely declined after 10 minutes from the onset of ischemia (43.9% of the control values). Subsequently, the level of 5-HT remained depressed until 360 minutes after occlusion. The 5-HIAA content rose after the ischemic insult, reaching a maximum by 60 minutes following MCA occlusion (greater than the control values).

Some studies have investigated the levels of biogenic amines in postischemic periods following the release of carotid occlusions in gerbils. Gaudet et al., (1978) investigated the effect of re-circulation after short periods of occlusion (5 minutes, 30 minutes, 60 minutes) on 5-HT levels in the cerebral cortex of gerbils. Bilateral hemispheric 5-HT reduction was measured in control and stroked animals after only 5 minutes of occlusion. 5-HT remained depressed after 30 and 60 minutes of occlusion except in the non-occluded hemisphere of non-stroked animals. During recirculation, the 5-HT level remained the

same as the baseline measurement. Mrsulja et al., (1976) reported that in animals occluded for 15 minutes, there were no differences in content of 5-HT between left (ischemic) and right (control) cerebral hemispheres. However, the 5-HT level one hour after onset of ischemia was significantly increased on the ischemic side and remained elevated even in animals sacrificed after six hours of occlusion. The 5-HT levels at 1 hour after release from occlusions of 15 minutes or 1 hour duration were significantly increased, as compared to the values registered immediately upon termination of the occlusions. However, in animals occluded for longer periods, the peak levels were reached by the end of occlusion and values declined subsequently. It should be noted that these results, showing an increase in 5-HT in ischemic brain tissue, are at variance with most studies. In general, the 5-HT levels have been shown to decrease under similar experimental conditions. The authors had no clear explanation for this discrepancy.

iv) γ-aminobutyric acid

Lust et al., (1975) found that the levels of GABA in the ischemic cerebral cortex increased 2.5-fold after 1 hour post-ligation. Similarly, Jellinger et al., (1978) reported marked elevation of GABA in recent infarct human brains; in the necrotic area (about 120% of controls), and particularly in edematous regions (about 190% of controls). The increase was more pronounced in the white matter.

Mrsulja et al., (1978) studied alterations of neurotransmitters and enzymes during ischemia in gerbil cerebral cortex. They produced a transient bilateral carotid occlusion (up to 15 minutes). The levels of GABA increased and those of glutamate decreased during bilateral occlusion; glutamate content was reduced after 10 and 15 minutes, but

not after 5 minutes of ischemia. GABA, metabolizing enzymes (glutamate decarboxylase (GAD) and GABA - transaminase (GABA-T) were also affected by ischemia; the activity of GAD was increased, while that of GABA-T decreased. The authors speculated that the increased level of GABA might be due to increased formation by GAD and/or decreased degradation by GABA-T.

Both Lust et al., (1975) and Mrsulja et al., (1978) speculated that the increasing levels of GABA and decreasing levels of glutamate may reflect the degree of depressed excitability in the ischemic cerebral cortex. Mrsulja et al., (1977) measured the effects of repeated cerebral ischemia on metabolites and metabolic rate in gerbils. After 5 minutes of unilateral ischemia, there was no significant changes in any of the metabolites measured. Following 60 minutes of ischemia, concentrations of ATP and P-creatine were markedly reduced. Glucose and glycogen also decreased and there was a concomitant increase in lactate. GABA concentrations were increased 3-fold, and cyclic AMP 5-fold, the concentrations of GABA being substantially greater after 60 minutes than after 5 minutes of repeated ischemia, thus indicating a close relationship between depressed cerebral metabolism and GABA elevation.

Pulsinelli and Francis (1983) produced ischemic damage to striatal GABAergic neurons in Wistar rats, by permanent occlusion of the common carotid arteries for 20, 30 or 40 minutes. Striatal GAD activity was measured at 5 to 8 days after 20, 30 and 40 minutes of forebrain ischemia. Insignificant decrease in striatal GAD activity was noted after 20 minutes but significant depression of GAD activity was noted after 30 and 40 minutes of forebrain ischemia. Hippocampal GAD activity was not different from control values following 40 minutes of forebrain

ischemia and 5 to 8 days of post-ischemic survival. They suggested that GABAergic neurons as a neurotransmitter-defined population are not equally sensitive to ischemia throughout the brain.

Weinberger and Cohen (1982) measured GABA and glutamate in synaptosomes prepared from the ischemic and control cerebral hemispheres of gerbils after left carotid ligation. Significant decreases in uptake by synptosomes did not occur until 16 hours after carotid ligation. GABA uptake was reduced to 28% of control and glutamate to 47% of control. GABA uptake was also significantly reduced relative to glutamate. Strong et al., (1983) studied synaptosomal uptake of [^{3}H] GABA in samples from ischemic areas in baboons and cats subjected to middle cerebral artery occlusion. In baboons there was significant impairment of uptake in severely ischemic regions. No changes in uptake of GABA was seen in the regions with mild to moderate ischemia. In cats, there was a significant relationship between mean ischemic flow and GABA uptake.

Thus, impaired uptake of GABA, accompanied by marked increases in brain GABA levels appear to be a consistent feature of cerebral ischemia. The increased GABA level might be due to, 1) increased formation by GAD and/or 2) decreased degradation by GABA-T. GABAergic neurons are not equally sensitive to ischemia throughout the brain and there are differences in the GABA uptake depending on the severity of the ischemia.

CHAPTER III

A REVIEW OF RECENT EVIDENCE FOR THE EFFECTIVENSS OF OPIATE ANTAGONISTS

Naloxone Therapy

Holaday and Faden (1980) demonstrated the reversal of hypotension, hypothermia and hypoventilation in cervical spinal cord-transected Sprague-Dawley rats with use of naloxone, an opiate antagonist. They suggested that these effects of naloxone seem to be mediated by a blockade of endorphin effects at opiate receptors in the central nervous system. In a subsequent study Faden, et al (1981) traumatized the spinal cord of adult cats and treated them with naloxone. Contusion of the cervical spinal cord produced a transient pressor response exceeding 100 mm-Hg followed by a gradual decline of mean arterial pressure over the next 45 minutes to a value approximately 15 mm-Hg below trauma levels. Naloxone treatment significantly increased mean arterial pressure to a maximum between 5 and 15 minutes after treatment. Neurological recovery was also significantly better in naloxone-treated animals than in saline-treated controls at 1, 2 and 3 weeks. The differences in neurologic function between the groups were greatest at 1 week. They suggested that beneficial effects of naloxone result from its ability to secondarily improve local spinal cord blood flow and to block the opiate receptors which may contribute the pathophysiology of spinal cord injury.

The beneficial effects of naloxone in recovery from spinal cord injury prompted other investigators to assess its efficacy in the treatment of neurological dysfunction secondary to cerebral ischemia. Hosobuchi et al., (1982) reported a significant reversal of

ischemia-induced neurologic deficit in gerbils by naloxone. Following occlusion of the right common carotid artery, 59 of 140 gerbils (42 percent) manifested signs of stroke 2 to 4 hours after surgery; none developed stroke more than 4 hours after surgery. They reported that their gerbils recovered from anesthesia (40 mg/kg of pentobarbital) and were alert and responsive within 2 hours. Fifteen of asymptomatic gerbils received no treatment were used as controls. Saline injection did not change the neurological status up to 9 hours after ligation. In animals injected with 1 mg/kg of naloxine hemiparesis or circling behavior or both invariably disappeared within 3 to 10 minutes (mean, 5.4 minutes). The naloxone effect lasted 20 to 30 minutes and repeated injection of naloxone consistently reversed all neurologic deficits. However, all ten gerbils were dead within 48 hours. The other 58 percent of gerbils did not develop any neurological deficits. Intraperitoneal injection of morphine sulfate (5 to 30 mg/kg; median dose, 15 mg/kg) within 3 to 20 minutes (mean, 8 minutes) induced a left hemiparesis that lasted 4 to 24 hours (mean, 18 hours) in 21 out of 24 gerbils. The morphine-induced stroke symptoms were reversed within 5 minutes in 10 out of 10 gerbils by intraperitoneal injection of naloxone. Brains from several untreated symptomatic gerbils analyzed for concentrations of immunoreactive β-endorphine-like material, demonstrated 40 to 80 percent higher levels in the ischemic right hemisphere than in the left control hemisphere. The authors hypothesized that cerebral ischemia causes an increase in the level of β-endorphine that in turn, by an unknown mechanism, produces hemiparesis. If there is no cerebral infarction (i.e. irreversible damage), this effect can be reversed by naloxone.

Similarly, Hosobuchi, et al (1982) administered naloxone (0.4 mg i.v.) in a double-blind manner to 2 patients with neurolgical deficits following stroke. The neurological deficits rapidly but temporarily improved.

Holaday and D'Amato (1982) attempted to replicate the experimental results reported by Hosobuchi and his colleagues. They evaluated functional neurologic recovery in gerbils that had been subjected to 30 minutes of bilateral carotid ligation. Sixty percent of the gerbils were dead within 8 hours. (80 percent dead within 3 days) regardless of drug treatment. They found improved cardiovascular and respiratory function after naloxone but no improvement in neurological status or survival time. Since they did not see any reversal of neurological status in gerbils with temporary bilateral occlusion, they further investigated the effect of naloxone in gerbils subjected to permanent unilateral carotid occlusion. The animals were randomly divided into groups that received saline or naloxone. Naloxone did not improve the neurologic scores or the mortality. However, morphine exacerbated the neurological deficits and increased the mortality. Seventy percent of the gerbils treated with morphine showed severe neurologic impairment and died within 7 days. They concluded that naloxone has no therapeutic effect on cerebral ischemia in the gerbil.

Levy et al., (1982) used nuclear magnetic resonance (NMR) imaging to evaluate the intracerebral changes over time in gerbils following unilateral carotid artery ligation. Despite reports that naloxone reverses the neurologic deficits seen in animals following acute cerebral ischemic lesions, their preliminary results failed to demonstrate an effect of naloxone on any of the NMR image parameters

evaluated. Upon comparison of the 2 mg/kg naloxone i.p., no significant differences between the pre-naloxone and post-naloxone conditions were observed at any time point over the 24 hour testing period.

Thus, attempts to demonstrate a beneficial effect of naloxone in the treatment of cerebral ischemia have produced conflicting results. It seems reasonable to expect that any agent which produces rapid reversal of neurological dysfunction would initiate a normalization of metabolism and neurotransmission in the affected brain tissue.

CHAPTER IV

METHODS

A. INDUCTION OF CEREBRAL ISCHEMIA

Seventy-two adult male mongolian gerbils (meriones unguiculatus) weighing 60 to 70 grams were studied. Animals were caged at a constant temperature in simulated day and night conditions and were allowed free access to drinking water and chow. The gerbils were anesthetized with thiopental sodium (pentothal) (20 mg/kg) and halothane (2% in oxygen).

The right common carotid artery was exposed through a ventral midline incision with the aid of a stereoscopic microscope. The artery was carefully dissected free of its accompanying vagus nerve, jugular vein, and coagulated with microbipolar forceps and transected. The animals were returned to individual cages for subsequent observation and neurological assessment.

B. NEUROLOGICAL ASSESSMENT

Following recovery from anesthesia, the gerbils were observed for the initial movement, and subsequently for behavioral signs of ischemic-induced neurological deficits. Animals that exhibited ptosis, diminished limb movement, splaying of limbs, hemisensory neglect and ipsilateral circling with head and neck deviation were assigned to the neurological deficit group. Animals that exhibited abnormal motor behavior, i.e. wild running, focal-clonic limb movement, rolling fits and tonic-clonic seizure were placed in the seizure group (Welch et al 1978). Those animals which did not show any neurological signs except ptosis were placed in the asymptomatic group. Behavioral changes were recorded as present (+) or not present (-) as they occurred. The

interval between carotid division and onset of symptoms was recorded (Table 1).

The type of drug treatment was determined by presence or absence of neurological dysfunction two hours after carotid occlusion. However, onset of seizure activity at earlier times resulted in those animals being assigned to the seizure group. The experimental protocol is summarized in the flow chart below.

Flow Chart

Anesthesia

R Common Carotid Ligation n=72 | Sham Operated n=10

(N.D.G.) n=25 | (Seizure Group) n=20 | (A.S.N.D.G.) n=27

3 hr.

Saline n=12 | Naloxone n=13 | Saline n=10 | Clonidine n=10 | Saline n=13 | Morphine n=14

Sacrifice for brain assays

Animal groups:

1. Neurological deficit group (I.N.D.G.)
2. Seizure group
3. Asymptomatic of neurological deficit group (A.S.N.D.G.)

Sham operations were performed on 10 gerbils which were randomly selected from cages. Identical anesthesia, midline cervical incision and exposure of the right common carotid artery was performed.

C. Drug therapy

The following present the drugs which were used and the dose and timing of the drug injections.

Drugs Used	Dose	Timing of Injection
Naloxone	1.0 mg/kg I.P.	20 min before sacrifice
Morphine	15.0 mg/kg I.P.	60 min before sacrifice
Clonidine	0.4 mg/kg I.P.	immediately after development of seizure activity

Each drug was transfered to a sterile injectable vial. Then a placebo vial was prepared with an equal volume of saline. Each vial was labelled blindly to the experimenter, e.g. naloxone (1) 1 mg/kg I.P. or naloxone (2) 1 mg/kg I.P.

Following injection of either drug or saline in random order, animals were observed for their neurological changes until 3 hour after carotid ligation when all animals were sacrificed (Table 1).

D. Removal of brain

Immediately after cervical dislocation, animals were decapitated, scalp and underlying faciae were rapidly cut open. Exposure of the brain was accomplished by removing bone with a small bone cutter. Extreme care was taken to prevent injury to the brain tissue. Following removal of the dura and sectioning of the spinal cord and cranial nerve attachments, the brain was removed and placed in isopentane over dry ice. The total time for removal of the brain was between 1-2 minutes.

E. Biochemical analysis

The biochemical analysis of the brain was performed in double blind manner. After removal of the cerebellum and pons-medulla (a scalpel cut was made transversely through the upper midbrain), the remainder of the brain was cut vertically along the midline between the two hemispheres. The two halves were weighed, and each was homogenized in 5 volumes of a

TABLE 1

Gerbil Stroke Model

Date:_____ Animal #:_____ Weight:_____

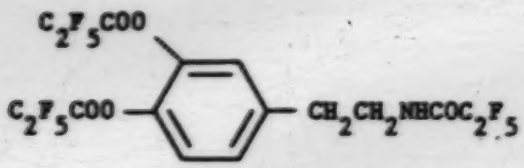
					Neurological deficit					Seizure							
Time	Anesthetic	(R) Carotid ligation	Initial Movement	ptosis	Dimished limb mov. R/L	Splaying of limb: R/L	Hemisensory neglect	Ipsilateral circling with head and neck deviation	Wild running	Focal-Clonic limb movement	Rolling fits	Tonic-clonic seizure	Coma	Drug administration	Death/Removal of the brain

COMMENTS:

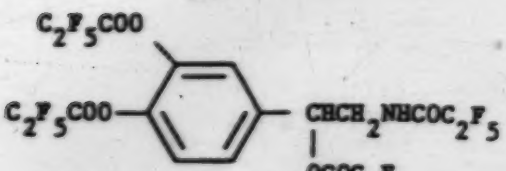
mixture of methanol/5N HCL, 9/1. After centrifugation, the supernatant was retained and 300 µl was utilized for analysis. Tranylcypromine (20 µl) was added to act as internal standard. The supernatant was taken to dryness under a stream of nitrogen. To the residue was added 25 µl of ethyl acetate and 50 µl of pentafluoropropionic andydride (PFPA). After heating at 60° for 30 minutes, the screw-cap glass tubes containing the reaction mixture were left at room temperature for 5 minutes. Hexafluoroisopropanol (HFIP) (100 µl) was added, and the tubes were left standing at room temperature for a further 30 minutes. The reagents and ethyl acetate were removed under a stream of nitrogen, and the residue was taken up in cyclohexane (800 µl). An aliquot of the mixture in cyclohexane was injected on to a gas chromatogarph (GC) equipped with an OV-1 12 m fused silica capillary column and an electron capture detector.

GC conditions: A Hewlett-Packard Model 5880 GC was employed. The oven temperature was programmed to increase from 80° to 270° at a rate of 8°/min. The carrier gas was helium at a flow rate of 2 ml/min. The makeup gas at the detection was argon/methane, 95/5, at a flow rate of 35 ml/min. The injection port temperature was 250°, and the detection temperature was 300°.

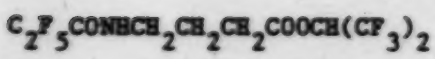
Derivatives of NE, DA, and GABA: PFPA reacts with amine, phenol and alcohol groups, while HFIP reacts with carboxylic acid groups (Koslow et al, 1972; Davis et al, 1977). Therefore, the stuctures of the final derivatives are shown below:



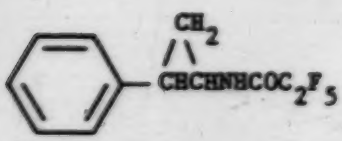
DA



NE



GABA



TRANYLCYPROMINE

Structures of the derivatives were confirmed using combined gas chromatography-mass spectrometry (GC-MS).

Retention times of the derivaties: GABA - 4.4 min, tranylcypromine - 9.8 min, NE - 11.9 min, and DA - 12.4 min.

Calculation of amounts of amines present: Standard curves were constructed by running known amounts of standards of GABA, NE and DA in 300 µl of MeOH/HCl solution through the same procedure as the brain

extracts. Tranylcypromine (20μg) was also present in these samples. The peak height ratios (i.e., GABA/TCP, NE/TCP and DA/TCP) were determined and plotted against the amounts of GABA, NE and DA present. The peak height ratios from the brain samples were calculated and by comparing to the values on the standard curve, the amounts of GABA, NE and DA were determined.

Statistical Methods

The behavioral data were analyzed by means of chi-square tests on the frequencies of animals showing either an improvement, a worsening or no change in their neurological status. The neurochemical data were analyzed by analyses of variance with groups as one factor and hemisphere as a second (repeated) factor. Where necessary, multiple comparisons were made using t-tests calculated using the appropriate error term from the analysis of variance in place of S^2 in the denominator of the t ratio. Since multiple t-tests increase the probability of making a type I error, Dunn's correction to the level of significance was employed. This correction involves dividing α by the number of groups involved in the multiple comparisons (Dunn, 1961).

CHAPTER V

RESULTS

A. CONTROL GROUPS

There were 4 groups of animals which were used as controls; a sham-operated group and three saline groups from the drug experiments. Following carotid ligation, animals were divided into 3 groups based on their neurological evaluation. Animals with seizure symptoms (wild running, focal-clonic climb movement, rolling fits, tonic clonic seizure) were assigned to the seizure group. Animals without seizure but with neurological deficit (ptosis, diminished limb movement, splaying of limb, hemisensory neglect and ipsilateral circling with head and neck deviation) were assigned to the neurological deficit group. Animals without any neurological symptoms except ptosis were assigned to the asymptomatic group.

1. Behavioral Data

The mean time period between surgery and onset of initial movement between groups varied depending on their neurological status; sham $\bar{X}$ = 9.8 min, seizure group $\bar{X}$ = 22.7 min, asymptomatic group $\bar{X}$ = 13.8 min, stroke group $\bar{X}$ = 12.9 min. There was a greater delay in the onset of initial movement in the seizure group (Table 2).

Neurological status was compared prior to the saline injection and just before sacrifice and the animals were divided into three groups: improved, worse or no change based on the number of observed neurological deficits. Eighty percent of seizure group animals became worse and 20 percent showed no change. Asymptomatic animals did not show any changes with saline injection. There was no neurological

Table 2

Average time interval between surgery and inital movement

Group	Treatment	
Seizure	Saline	$\bar{X}$ = 22.7 min
	Clonidine	$\bar{X}$ = 20.1 min
Asymptomatic	Saline	$\bar{X}$ = 13.8 min
	Morphine	$\bar{X}$ = 15.4 min
Neurological Deficit	Saline	$\bar{X}$ = 12.9 min
	Naloxone	$\bar{X}$ = 13.9 min
Sham Operation		$\bar{X}$ = 9.8 min

status change in 58 percent of the neurological deficit group, 8 percent became worse and 34 percent showed some improvement in their neurological status (see Table 3).

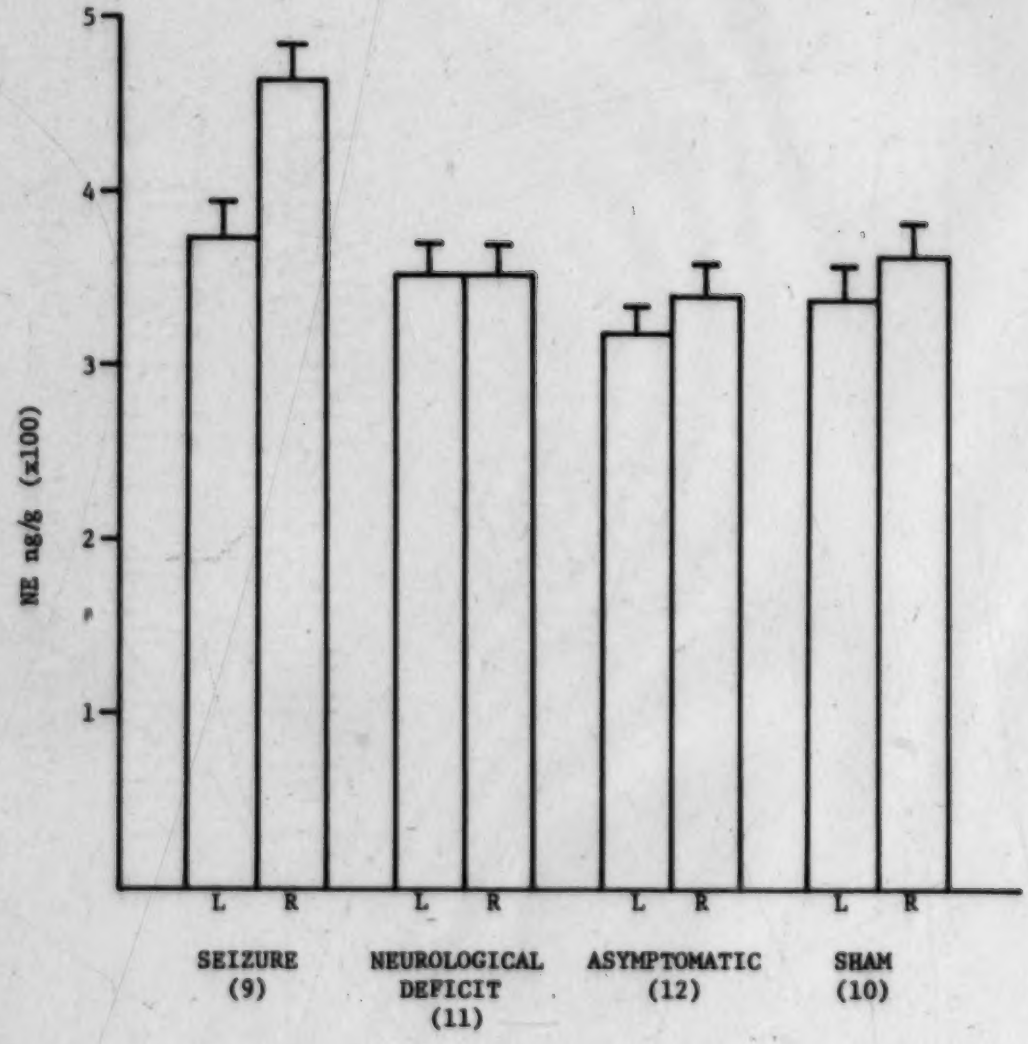
2. Effect of ischemia or neurotransmitters

1) Norepinephrine

Figure 1 shows the NE level in the saline groups with different neurological status and the sham operated group. Although there were only slight increases in NE level in the right hemisphere of the sham, operated asymptomatic and neurological deficit groups, there was substantially more NE in the right than the left hemisphere of the seizure group. There were similar NE values for the left non-ischemic hemispheres in all groups. The analysis of variance (ANOVA) showed that, overall, there was a significant difference between the two hemispheres ($F(1,38)=6.83$, $p<.05$), but the differences between groups was not statistically significant ($F(3,38) = 1.52$, $p>.05$). The interaction between groups and hemisphere was also not significant ($F(3,38) = 1.95$, $p>.05$) which means that the difference in NE level between the two hemispheres did not vary significantly between groups. Nevertheless, t tests suggest that there were some significant interaction effects. The difference between the hemispheres was significant for the seizure group $t(38) = 4.463$, $p<.001$) but not for the other groups. Moreover, t-tests between groups on the differences between the hemispheres indicated that the hemisphere difference for the seizure group was significantly greater than the hemisphere difference for the neurological deficit group ($t(38) = 3.300$, $p<.01$). Similar comparisons between the seizure groups and the asymptomatic and sham operated groups were not significant ($t(38) = 2.507$, $p<.05$; $t(38)$

FIGURE 1

Relationship between neurological status and norepinephine (NE) levels (mean $\pm$ SEM) in the left (L) and right (R) hemispheres of gerbils subjected to right carotid occlusion. Levels in sham operated animals are shown for comparison. Number of animals in parentheses.


NE ng/g (x100)
5
4
3
2
1
L
R
SEIZURE
(9)
NEUROLOGICAL
DEFICIT
(11)
ASYMPTOMATIC
(12)
SHAM
(10)

=2.323, $p<.05$ respectively).

ii) Dopamine

Figure 2 shows that while the seizure group had higher DA levels in the left hemisphere, the other groups had higher DA levels in the right hemisphere. In the ANOVA, there was a significant hemisphere by group interaction ($F(3,38) = 4.17$, $p<.05$) which indicates that the difference between hemispheres is significantly different between groups.

iii) γ-aminobutyric acid

Figure 3 shows that there were increased levels of GABA in the right hemispheres of all groups while the left hemisphere GABA levels were similar in all groups. The GABA level in the right hemisphere tended to increase with the severity of the neurological symptoms. In the ANOVA, there was a significant hemisphere effect ($F(1,38 = 33.71$, $p<0.001$) and also there was a significant group effect ($F(3,38) = 5.3$, $p<0.01$) which means that the right hemisphere and the left hemisphere GABA levels significantly differ and there were significant differences in GABA levels between groups. However, the hemisphere by group interaction was not significant ($F(3,38) = 2.83$, $p = .051$), which means that the difference in GABA level between the two hemispheres did not vary significantly between groups. Nevertheless, t-tests suggest that there were some significant interaction effects. The right and left hemisphere difference was greater for the seizure group then for asymptomatic ($t(38) = 2.86$, $p<0.05$) or sham ($t(38) = 4.003$, $p<.01$) groups.

B. DRUG TREATMENT GROUPS

Following carotid ligation, the drug treated animals were observed for the onset of initial movement and development of neurological

FIGURE 2

Relationship between neurological status and dopamine (DA) levels (mean ± SEM) in the left (L) and right (R) hemispheres of gerbils subjected to right carotid artery occlusion. Levels in sham operated animals are shown for comparison. Number of animals in parentheses.

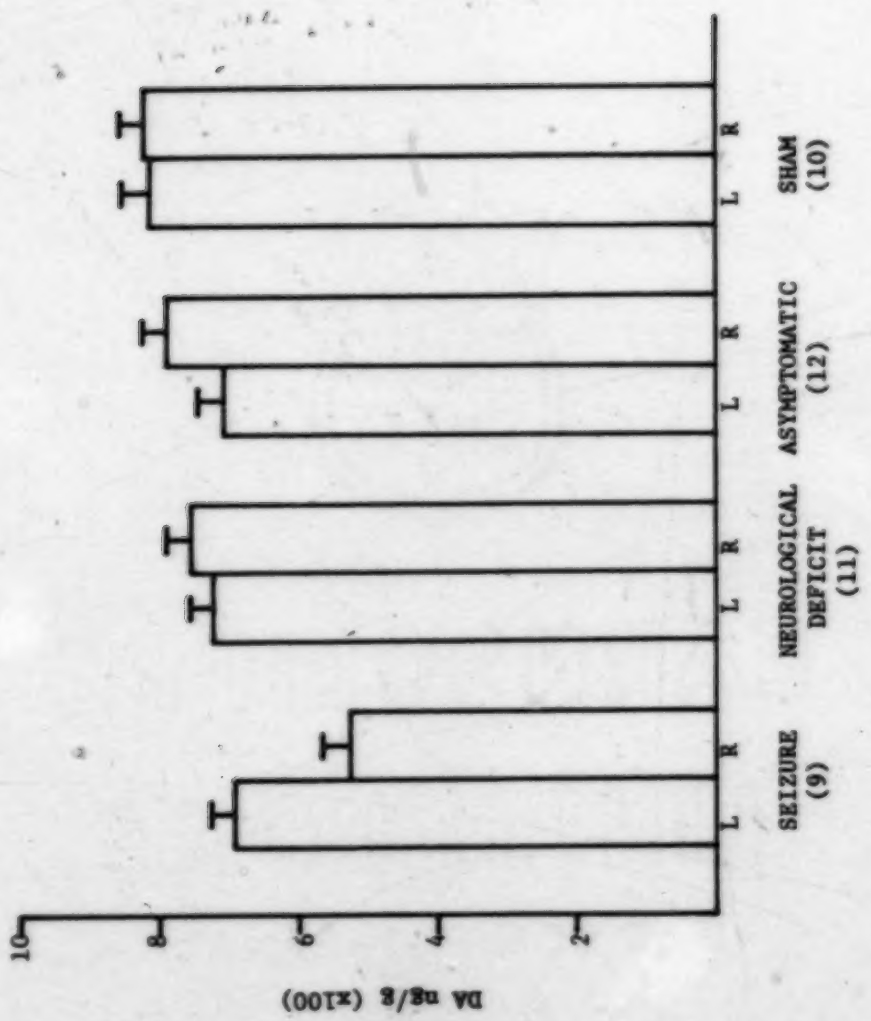
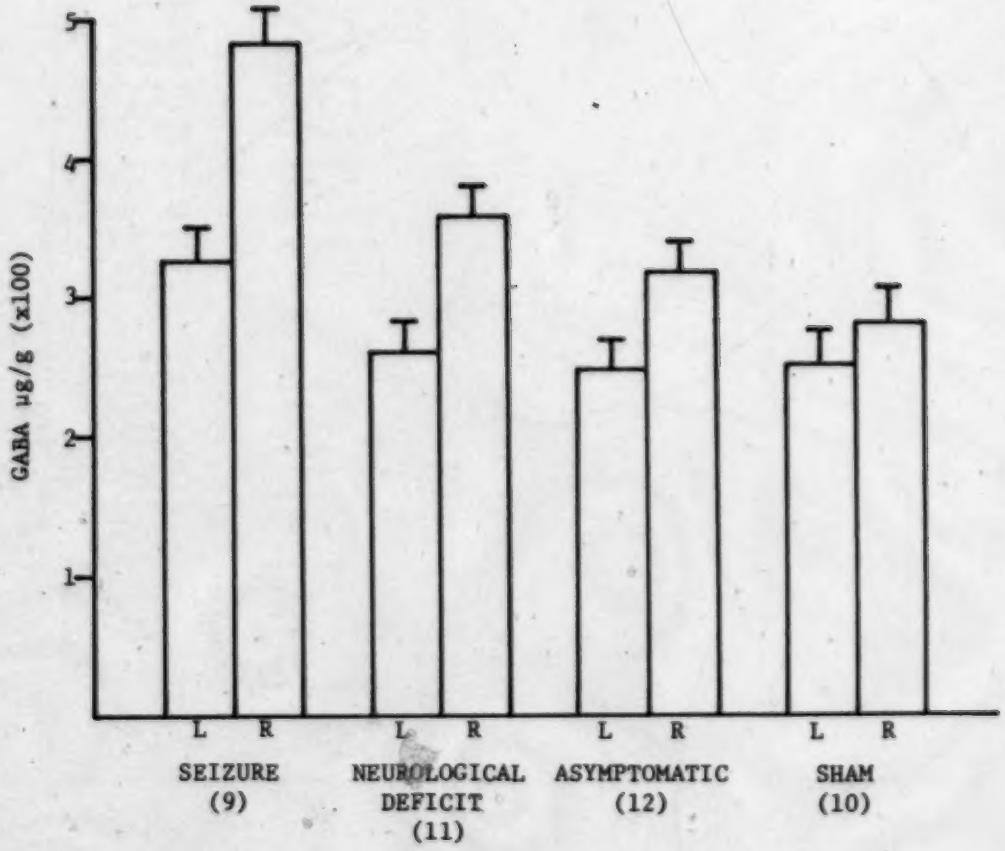

DA ng/g (x100)
10
8
6
4
2
L
R
SEIZURE (9)
NEUROLOGICAL DEFICIT (11)
ASYMPTOMATIC (12)
SHAM (10)

FIGURE 3

Relationship between neurological status and γ-aminobutyric acid (GABA) levels (mean ± SEM) in the left (L) and right (R) hemispheres of gerbils subjected to right carotid occlusion. Levels in sham operated animals are shown for comparison. Number of animals in parentheses.


GABA µg/g (x100)
5
4
3
2
1
L R
L R
L R
L R
SEIZURE (9)
NEUROLOGICAL DEFICIT (11)
ASYMPTOMATIC (12)
SHAM (10)

manifestations. Depending upon their symptoms, they were assigned to the seizure group, asymptomatic group or neurological deficit group and treated with clonidine (0.4 mg/kg), morphine (15 mg/kg), naloxone (1 mg/kg) respectively. All drugs were administered intraperitoneally.

1. Behavioral data

The mean time period between carotid ligation and the onset of initial movement between groups varied depending on their neurological status (seizure group = 20.1 min, asymptomatic group = 15.4 min, stroke group = 13.9 min). There was a great delay in the onset of initial movement in the seizure group. Neurological status was compared prior to drug injection and just before sacrifice and the animals were divided into three groups; improved, worse or no change. Table 3 shows one hundred percent of seizure group improved after clonidine injection $\chi^2(2) = 20.0$, $p<0.001$) whereas asymptomatic animals developed stroke symptoms after morphine injection. ($\chi^2(1) = 23.14$ with Yates correction $p<.001$. However, naloxone did not have any effects on animals with neurological eficits. ($\chi^2(2) = 1.3$, $p>.05$). Tables 4, 5 and 6 show the frequency of particular neurological symptoms before and after treatment with saline, clonidine, morphine and naloxone.

2. Effect of drug treatments on neurotransmitter

i) Norepinephrine

Figure 4 shows that the level of NE in each hemisphere of drug-treated groups and saline-injected groups. Although there seems no difference between saline versus morphine groups and saline versus naloxone groups, clonidine versus saline groups showed a trend for differential effects in each hemisphere. In the ANOVA, there was a significant interaction between hemispheres and groups ($F(1,16) = 13.08$,

Table 3

Effect of drug treatments on neurological status in gerbils with seizures, neurological deficits and no symptoms following carotid occlusions.

	Seizure		Asymptomatic		Neurological Deficit	
	Saline	Clonidine	Saline	Morphine	Saline	Naloxone
Improved	0	10	0	0	4	6
Worse	8	0	0	14	1	2
No Change	2	0	13	0	7	5
Total	10	10	13	14	12	13
	χ^2= 20.0 P<.001*		χ^2= 23.14 with Yates collection P<.001		χ^2= 1.028 P>.05	

* The chi-square test was used to compare the frequencies within the Improved, Worse and No Change categories produced by saline or drug treatement.

TABLE 4

Frequency of post-ischemic seizure symptoms before and after treatement with saline placebo and clonidine.

	Saline (n=9)		Clonidine (n=9)	
Symptoms	Pre	Post	Pre	Post
Wild running	5	7	4	0
Focal-clonic	10	9	9	3
Rolling	8	9	9	0
Tonic-clonic	7	9	6	0

TABLE 5

Frequency of post-ischemic neurological symptoms before and after treatement with saline placebo and morphine

	Saline (n=12)		Morphine (11)	
Symptoms	Pre	Post	Pre	Post
Ptosis	5	5	6	6
Diminished limb movement	0	0	0	0
Splayed hing limb	0	0	0	0
Hemisensory neglect	0	0	0	2
Ipsilateral circling	0	0	0	10
Wild running	0	0	0	1

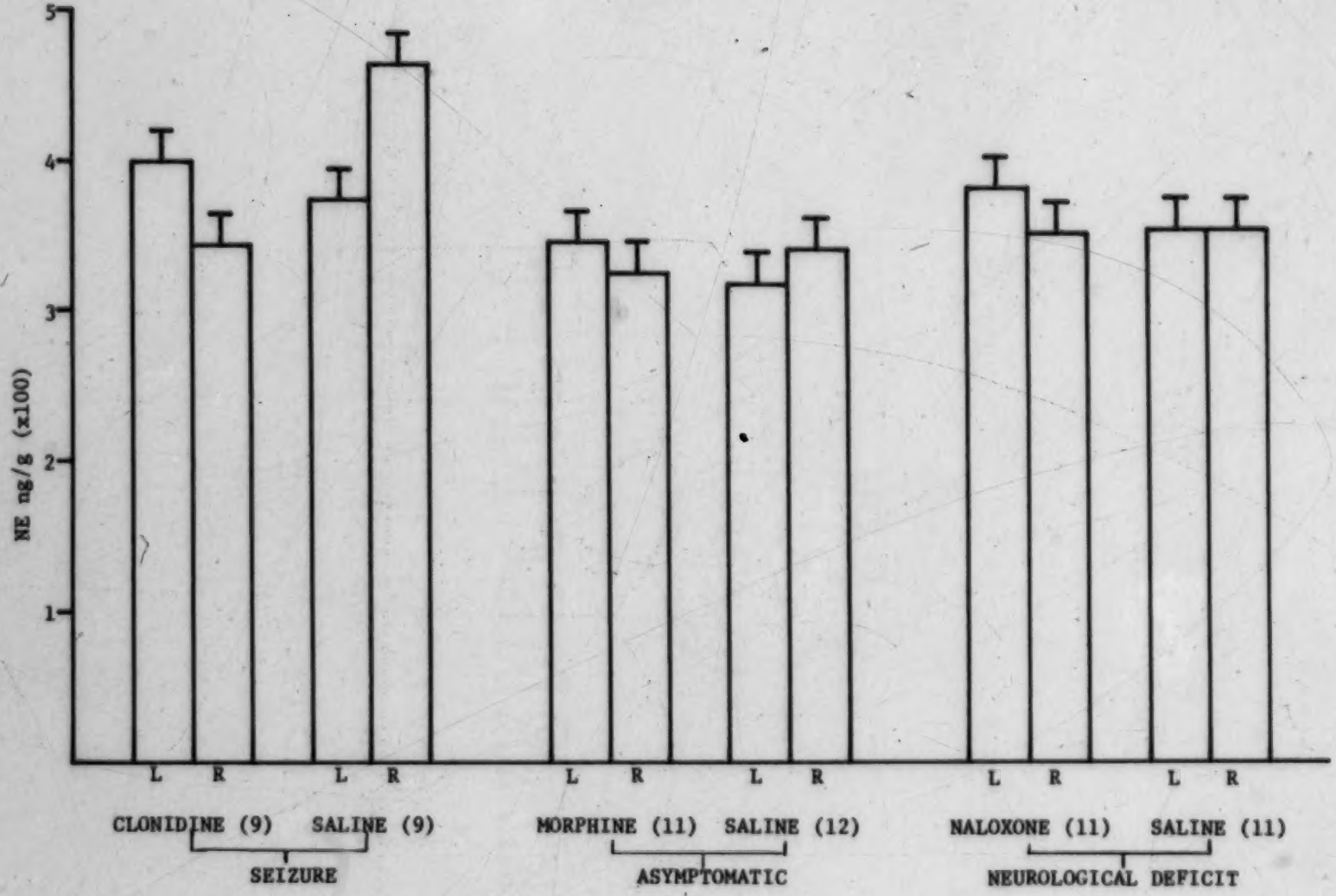
TABLE 6

Frequency of post-ischemic neurological symptoms before and after treatment with saline placebo and naloxone

	Saline (11)		Naloxone (11)	
Symptom	Pre	Post	Pre	Post
Ptosis	7	9	9	8
Diminished limb movement	5	3	5	1
Splayed hind limb	4	2	4	1
Hemisensory neglect	1	1	2	0
Seizure symptoms	0	3	0	4

FIGURE 4

Effect of drug treatment on norepinephrine (NE) levels (mean $\pm$ SEM) in the left (L) and right (R) hemispheres of gerbils subjected to right carotid occlusion. Number of animals in parentheses.


NE ng/g (x100)
5
4
3
2
1
L R L R
L R L R
L R L R
CLONIDINE (9) SALINE (9)
MORPHINE (11) SALINE (12)
NALOXONE (11) SALINE (11)
SEIZURE
ASYMPTOMATIC
NEUROLOGICAL DEFICIT

$p<.01$) which means that clonidine had significantly different effects in the two hemispheres. This interaction appears to be due primarily to clonidine producing a large reduction in right hemisphere NE with only slight changes, if any, in the left hemisphere.

ii) Dopamine

Figure 5 shows the levels of DA for the drug group and the saline injected groups in each hemisphere. Administration of clonidine resulted in an increase in the level of DA in both hemispheres in the seizure group. However, the ANOVA demonstrated only that, overall, there was significantly higher levels of DA in the left hemisphere than in the right, ($F(1,16) = 16.47$, $p<.01$), which does not relate to clonidine effect. The other effects were not significant. In the asymptomatic group, morphine produced a decrease in the level of DA in the right hemispheres with little effect on the left hemisphere. In the ANOVA, while there was no significant differences between hemispheres or groups, the significant interaction ($F(1,21) = 15.9$, $P(.001$) indicates that morphine had significantly different effects in the two hemispheres, producing a decline in DA in the right hemisphere and little change in the left. In the neurological deficit group of animals, the effect of naloxone was surprisingly similar to morphine but the ANOVA did not show a significant interaction ($F(1,20) = 2.79$, $p = .110$).

iii) γ-aminobutyric acid

Figure 6 shows the levels of GABA in each hemisphere of drug treated groups and saline injected groups. The right hemisphere had greater levels of GABA in all groups. The clonidine treated right hemisphere had a substantially larger GABA level compared to morphine or

FIGURE 5

Effect of drug treatments on dopamine (DA) levels (mean ± SEM) in the left (L) and right (R) hemispheres of gerbils subjected to right carotid artery occlusion. Number of animals in parentheses.

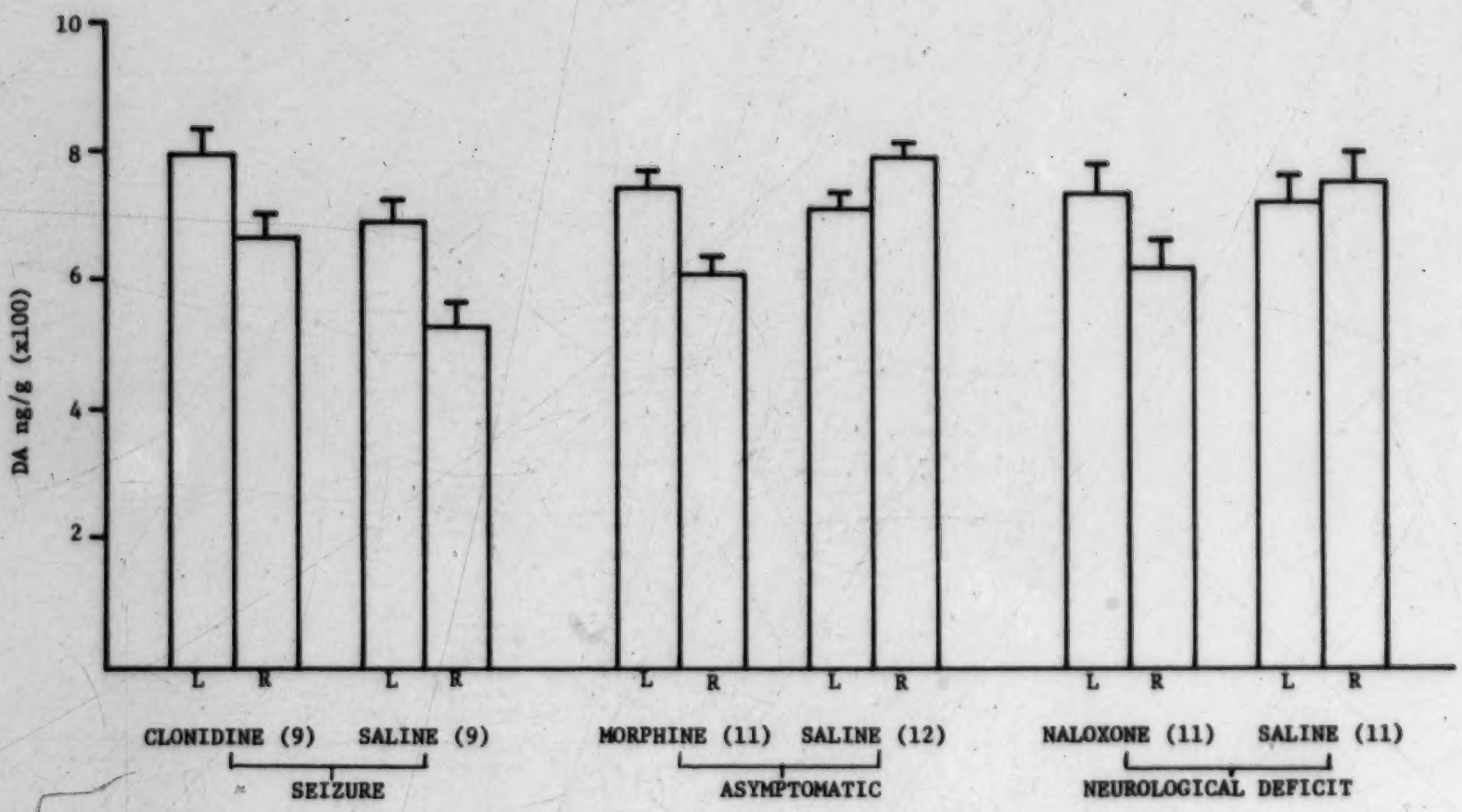
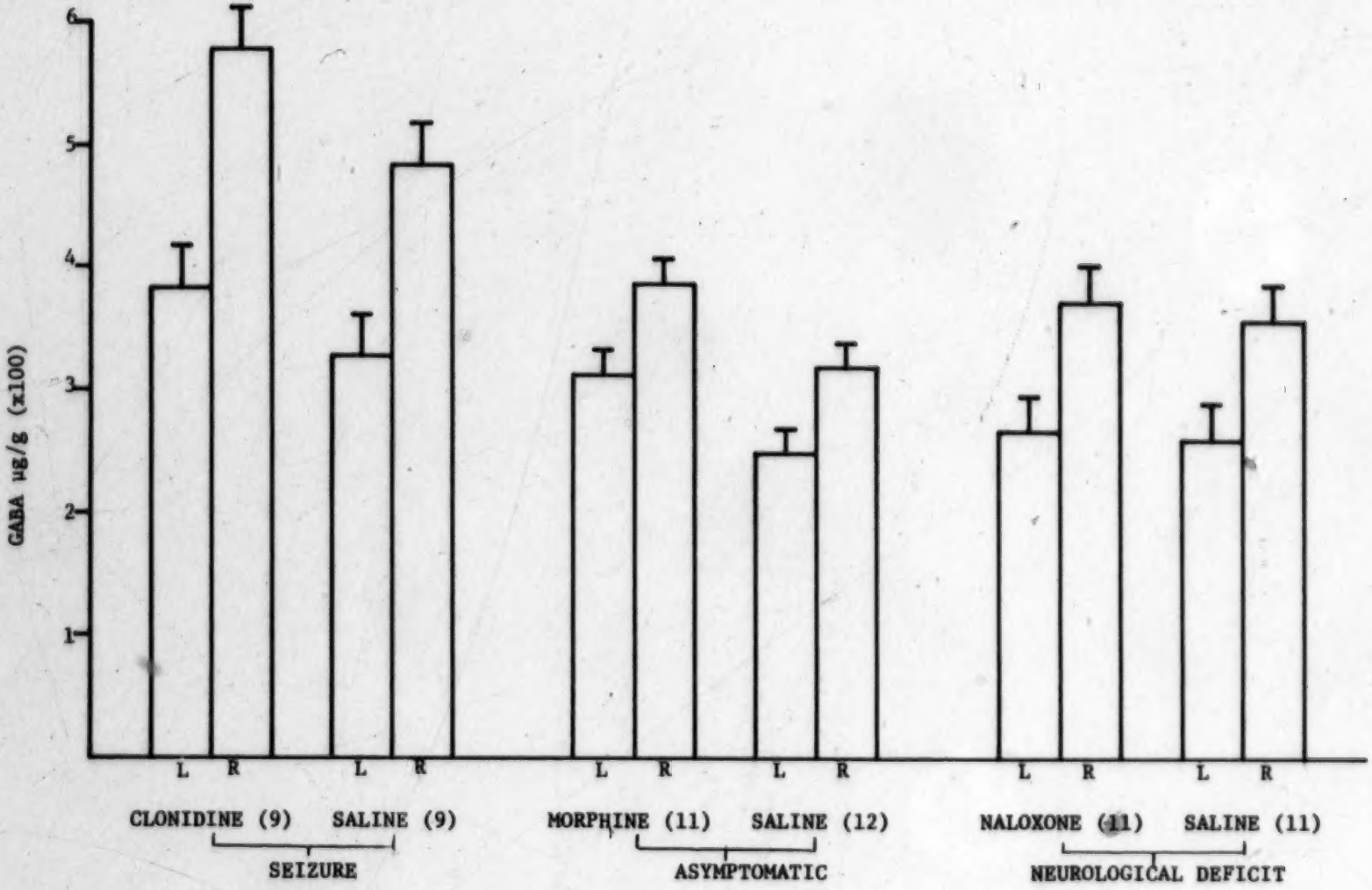

10
8
6
4
2
DA ng/g (x100)
L R L R
L R L R
L R L R
CLONIDINE (9) SALINE (9)
MORPHINE (11) SALINE (12)
NALOXONE (11) SALINE (11)
SEIZURE
ASYMPTOMATIC
NEUROLOGICAL DEFICIT

FIGURE 6

Effect of drug treatments on γ-aminobutyric acid (GABA) levels (mean ± SEM) in the left (L) and right (R) hemispheres of gerbils subjected to right carotid artery occlusion. Number of animals in parentheses.


GABA µg/g (x100)
6
5
4
3
2
1
L R L R
L R L R
L R L R
CLONIDINE (9)
SALINE (9)
SEIZURE
MORPHINE (11)
SALINE (12)
ASYMPTOMATIC
NALOXONE (11)
SALINE (11)
NEUROLOGICAL DEFICIT

naloxone treated groups. However, in the ANOVA, there was only a hemisphere effect ($F(1,16) = 28.7$, $p<.001$) which means GABA levels were significantly higher in the right hemisphere. Similarly morphine and naloxone showed only hemisphere effects ($F(1,21) = 16.18$, $p<.001$; $F(1,20) = 13.37$, $p<.01$ respectively) which means that the right hemisphere had greater level in all groups regardless of drug treatements. Consequently, drug treatments did not significantly affect GABA levels.

In summary, under control conditions, there were slight increases in the NE levels of the right hemisphere compared to left hemisphere in all groups of animals. The right hemisphere NE level in the seizure group was significantly higher than in the neurological deficit group. While the seizure groups had higher DA levels in the left hemisphere, the other groups had higher DA levels in the right hemisphere. The GABA level in the right hemisphere was greater in all groups and its level increased with the severity of the neurological defficits.

Under drug treatment conditions, there was a great delay in the onset of initial movement in the seizure group than other groups. Clonidine significantly improved seizure symptoms and morphine significantly worsened the neurological symptoms; however, there was no significant neurological change after injection of naloxone. In the neurochemical analysis, clonidine seemed to inhibit the NE increase in the right hemisphere of the seizure group while it seemed to produce little change in the NE level in the left hemisphere. Although clonidine seemed to increase the level of DA in both hemispheres, the ANOVA showed only a significant difference between hemispheres in both clonidine and saline treated animals. Morphine had a significant

interaction effect between hemispheres and groups which indicated that morphine had different effects on the DA levels of the two hemispheres. Although there was a trend for a decreased level of DA in Naloxone treated animals, it failed to reach significance. The GABA levels were higher in the right hemispheres of all groups.

CHAPTER VI

DISCUSSION

The experimental study of cerebral ischemia demands animal models in which a known ischemic insult produces a consistent effect. In the past, the frequent lack of consistent results, even in the same animal species or strain, was a major difficulty. Equally important, most experimental models based on interruption of arterial blood supply have required lengthy surgical procedures, thus making large-scale studies impractical. In 1966 Levine and Payan reported that mongolian gerbils, unlike many other animals, often develop brain infarction following unilateral ligation of the common carotid artery. This discovery paved the way for reliable processing of large numbers of gerbils for collecting statistically valid data based on various experimental parameters. Silberstein et al., (1978) stated that of all models, the gerbil stroke model is the most suitable mode for simulation "of local ischemia of the human brain". Skepticism must prevail in extrapalating animal test results to human clinical use, however many researchers using the gerbil stroke model have observed some close correlation that signal significant potential for eventual clinical application.

Experimental Methodology:

Kahn (1922) described the course of cerebral infarction in gerbils including the development of neurological symptoms such as hemiparesis (paucity of movement on one side of the body, a splayed-out hind limb when walking, external rotation of the paretic hind limb, asymmetry in extension of digits with loss of tone on the affected side, and circling behavior, and ptosis). The circling behavior was usually in a direction

ipsilateral to the side of the ischemia. The diameter of the circle varied and in 7 out of 16 symptomatic animals became progressively smaller until the animal rotated around the axis of their own bodies. Prior to death the animals became non responsive and finally lapsed into a comatose-like state. From his observation, it seems that the severity of the neurological deficit following the carotid ligation can be divided into 3 phases. The comatose state is more severe than the circling state which is more severe than the hemiparesis state. Harrison et al., (1979) retrospectively emphasized the importance of separating the animals according to the nature of the clinical deficit as previously reported by those described by Welch et al., (1978).

Interestingly, the occurrence of focal and generalized epileptic seizures in patients with carotid obstruction has been emphasized in the clinical literature since the observations of Moniz et al., (1937). Cacito (1982) investigated the occurrence of epileptic seizures among patients with MCA and carotid occlusive disease. Epileptic seizures occurred sometime during the clinical course of the disease in 17.3% of patients with carotid occlusion (which is approximately the same frequency as in the gerbil stroke model) and in 10.8% of patients with middle cerebral artery occlusion. Therefore, because seizures are an important symptom of ischemia the animals used in this study were carefully separated into 3 groups based on their neurological status: asymptomatic, neurological deficits and seizures. The neurological evaluation criteria were almost identical to those described by Welch et al., (1978) except that animals with ptosis alone were considered asymptomatic.

Ptosis does not seem to reflect cerebral infarction. Kahn (1972)

observed approximately 20% of animals with ptosis had no evidence of cerebral infarction. The frequent appearance of ptosis in both animals neurological deficit and asymptomatic animals was attributed by Berry et al., (1974) to interruption of the sympathetic supply during ligation of the common carotid artery. However, the appearance of a dry shrunken eye was thought to be likely due to interruption of external carotid blood supply to the contents of the orbit. Hosobuchi et al., (1982) also stated that ptosis reflects damage to the sympathetic nervous system, which is usually secondary to local carotid dissection. In my study, there were 2 animals in the sham group that had ptosis for a short period of time upon recovery from anesthesia. Therefore, those animals without any neurological symptoms except ptosis were considered as the asymptomatic group of animals. Overall, 72 gerbils were subjected to unilateral carotid ligation, and 45 (47%) had either seizure or stroke symptoms (neurological deficit); this rate is similar to that reported previously (Levy et al., 1976; Gaudet et al., 1977).

Pilot studies were done prior to deciding on the interval between ischemia and sacrifice (brain assay), the timing of drug injection, and the selection of anesthetics. During the pilot studies some animals were anesthetized with 40 mg/kg (intraperitoneal) of pentobarbital and others were anesthetized with 2% halothane. The halothane anesthetized animals recovered from anesthetic very rapidly, however, most developed seizure symptoms and eventually died. The pentobarbital group was quite variable in recovery time regardless of surgery (sham or carotid ligation), contrary to what Hosobuchi et al., (1982) have reported. They stated that their animals recovered from anesthesia (pentobarbital 40 mg/kg) within 2 hours and were alert and responsive. In the present

study it was necessary to have consistant baseline recovery time from anesthesia in order to differentiate the neurological symptoms from the anesthetic effects. Since the main objective of this study was to investigate the neurotransmitter levels in acute stage of cerebral ischemia, a short acting barbituate (Sodium Thiopental- 20 mg/kg) was administered in conjunction with 2% halothane. With this anesthetic procedure sham-operated animals showed initial movements within 10 min and behaved normally within 20 min from surgery. Analysis of the behavioral data from the untreated group of animals indicated a positive correlation between severity of neurological deficits and the mean time period for the initial movement post surgery (the seizure group > the stroke group > asymptomatic group > the sham group). However, it would be premature to generalize from these results without further studies.

The timing of drug injection was determined based on the duration of their effects. According to Hosobuchi et al., (1982), naloxone (1 mg/kg, i.p.) reversed hemiparesis within 5 to 10 min and lasted 20 to 30 min after which the deficit returned. Intraperitoneal injection of morphine sulfate (5 to 30 mg/kg; medium dose, 15 mg/kg) induced hemiparesis within 3 to 20 min that lasted 4 to 24 h (mean, 18 h). Therefore naloxone was injected 20 min prior to sacrifice and morphine was injected 1 h prior to sacrifice. Although there is no reported investigation of clinical or experimental cerebral ischemia which involved clonidine, the initial dose was determined based on other studies. The administration of clonidine, an antihypertensive -adrenergic agonist (Kobinger, 1978) produces several morphine-like behavioral effects, including analgesia (Lin et al., 1980) and relief of symptoms of opiate withdrawal in both animals and man (Fielding et al.,

1978; Gold et al., 1978). Clonidine's actions at the neuronal level also resemble those of morphine since both agents are known to reverse the emphasis of activity of central noradrenergic neurons precipitated by acute opiate withdrawal (Agajanion, 1978; Lavery and Roth 1980). The dose of clonidine used varied a great deal depending upon subjects and type of experiments (0.01-1.0 mg/kg). The final decision of the dose and timing of injection for the present study was based on pilot studies. The optimum level of clonidine seemed to be 0.4 mg/kg. It had to be administered immediately following the development of the seizure symptoms and was effective for 2 to 3 hours.

Neurotransmitter levels in the cerebral cortex of gerbils subjected to prolonged cerebral ischemia have been studied previously, (Lavyne et al., 1975a; Lust et al., 1975; Welch et al., 1977). The present study explored the effect of ischemia of short duration in order to examine if changes in neurotransmitter levels occurred which might contribute to events taking place at an early and critical stage of a developing infarct. The time period between carotid ligation and sacrifice of the animals was determined based on three requirements which were: 1) minimal or no development of brain edema, 2) sufficient time period to investigate drug effects, and 3) neurotransmitter changes free of possible anesthetic effects. During the pilot study, ischemic animals evidenced a "maturation period" during which the neurological status fluctuated for up to 60 min (with the exception of the seizure symptoms). Once animals developed seizure symptoms, they continued to have them without any improvement.

Berry et al., (1974) reported that histological changes depend upon the duration of carotid occlusion. The brain failed to reveal obvious

changes within the first 2 hr, but from 3 1/2 hrs on there were changes indicative of early necrosis with edema. During the very acute stage Mrsulja et al., (1976) found no difference in content of biogenic amines between ischemic and control hemispheres within 15 min of carotid ligation. Eventually, the NE and DA levels were reduced at 1 h after carotid ligation and at 3 hr the changes were pronounced. These results demonstrated that a 3 hr ischemic period would satisfy the requirements of the present study.

There are a number of investigators who have measured the level of catecholamines (NE, DA) and/or 5-HT in cerebral ischemia (for a review see Jellinger and Riederer, 1981). GABA levels were measured instead of 5-HT in this study. The size of the gerbil brain precludes doing regional brain assays and necessiates the use of highly sensitive and reliable equipment for neurotransmitter analysis such as the gas chromatography (GC) technique used in this study. There are a variety of techniques to detect compounds eluting from a GC. We used the electron-capture detector (ECD) which is a relatively selective detector and has the potential to detect as little as 1 pg of an organic compound containing an electrophoric substituent (Baker et al., 1982). Unfortunately, the assay procedure described here does not provide for simultaneous analysis of 5-HT. Given one aspect of this study (comparison of stroke vs seizure symptoms in cerebral ischemia), it seemed reasonable to measure GABA levels instead of 5-HT.

Untreated Groups

The results of the GABA measurements in the ischemic hemisphere are consistent with previous reports (Lust et al., 1975) showing marked increases. In the present study the GABA levels in the right hemisphere

tended to increase with the severity of the neurological symptoms (seizure > stroke > asymptomatic > sham) while the GABA levels in the left hemispheres were similar among groups. The NE and the DA levels found in this study are not entirely consistent with previous reports. In the present study, the NE levels were significantly higher in the ischemic hemisphere of the seizure group but not in the neurological deficit, asymptomatic or sham groups and the difference between the hemisphere in the level of NE was significantly greater in the seizure group than in the stroke group. The DA level in the ischemic hemisphere was unchanged except in the seizure group which had significalty lower DA level.

Lavyne et al., (1975) found large decreases in the NE and DA levels at 24 hrs post carotid ligation. Mrsulja et al., (1976) reported that, in gerbils, both the NE and DA levels were, significantly reduced at 3 and 6 hrs post carotid ligation. However, other investigators have reported differential changes for NE and the DA. Harrison et al., (1979) found only a tendency for lower NE level in the ischemic hemisphere and no consistant change in the DA level at 3.5 hrs post carotid ligation in gerbils. Gaudet et al., (1977) reported no change in the DA level but a large decrease in the NE level a 1 hr post carotid ligation and Zervas et al., (1974) stated that there was no change in the DA level up to 2 h but it was significantly reduced by 3 hr and 24 h post carotid ligation. However, the NE level was unaltered at 24 h post carotid ligtion.

There are numerous problems with these comparisons because of variation in anesthesia, surgical prepartion of animals, neurological assessment and brain assay techniques. However, one previous study

seems comparable to this study. Welch et al., (1978) investigated the contribution of post ischemic seizure activity to cortical monoamines. The anesthetized 86 gerbils with ether and ligated the right common carotid arteries. Upon recovery from anesthesia, the animals were classified as no stroke, stroke, or stroke plus seizure based on the neurological assessment. The animals were killed by direct immersion in liquid nitrogen at one and three hours after operation. Sham operated animals were treated the same as the experimental animals. They found that the levels of DA and NE in gerbils with and without signs of stroke did not differ from the control. In animals with stroke plus seizure, DA and NE levels were reduced solely on the occluded side at one hour, and were reduced bilaterally three hours after occlusion. Thus, Welch et al., (1978) found lower level of NE while we found higher level of NE in the ipsilateral hemisphere of seizure animals. The difference may be due to the different brain assay techniques used. In this study, neurotransmitter levels were measured with GC from entire hemispheres and compared while Welch et al., (1978) took samples of cerebral cortex from both hemispheres and analyzed them fluorometrically.

Although it was impossible to find a published report on the assay of brain during seizure, there is some clinical evidence which indicates increased release of NE during ischemia. Meyer et al., (1974) reported high levels of NE in the CSF of humans during the acute stage of cerebral ischemia which gradually declined over 3 weeks. There was a tendency for patients with more severe neurological deficit to have higher CSF concentration of NE compared to patients with less severe neurological deficits. Similar changes were observed in the plasma level of NE in stroke patients by Myers et al., (1981). They found a

significant increase in the plasma level of NE depending upon the severity of neurological deficit: intracerebral hemorrhage > seizure > cerebral infarction.

The influence of anesthesia on the level of catecholamines must be considered. The effect of transient ischemia on monoamine metabolism in the rat brain during 70% nitrous oxide and 150 mg/kg of phenobarbitone anesthesia was previously investigated (Calderini et al., 1978). They found a pronounced post ischemic decrease in NE and 5-HT in animals anesthetized with nitrous oxide but not in those given phenobarbitone. Since they used a transient, global ischemia model, their results may not be relevant but they indicate the importance of investigating the anesthetic difference while comparing the level of neurotransmitters in ischemia. There have been several studies which used diethyl ether, including Welch et al., (1978) and which reported decreased levels of NE during the acute stage of ischemia (up to 6 h post carotid ligation). Since this study employed a combination of 20 mg/kg thiopental and 2% halothane as the anesthesia, this may account for the difference in the neurotransmitter level.

Drug treated groups

In the seizure group, saline injection worsened the symptoms of 80% of the animals and clonidine injection improved 100% of animals. This improvement was correlated with the changes in the level of neurotransmitters. Clonidine injection was associated with prevention of an increase in NE levels in the ischemic hemisphere. Clonidine has not previously been used to attenuate seizure symptoms. If increases in the levels of NE are associated with seizure symptoms in patients, it might be possible to reduce such symptoms with clonidine. By means of

single unit recording techniques it has been found that a small systemically administered dose of clonidine inhibited the spontaneous firing of brain NE containing neurons in the locus coeruleus. In addition, the NE neurons were consistently inhibited by the direct application of minute amounts of clonidine. (Svensson et al., 1975; Aghajanian, 1978). Consequently it is possible that the reduced levels of NE produced by clonidine in the present study were due to the drug inhibiting the release of NE into ischemic hemisphere.

Increasing the level of GABA in the CNS protects animals against seizures (Kuriyama et al., 1966). This cannot be achieved by GABA or GABA precursors since they do not cross the blood brain barrier, but must involve the use of drugs which modify GABA synthesis or GABA metbolism. Such studies in animals have revealed a correlation between GABA levels in CNS synaptosomes (Wood et al., 1981) and in CSF (Loscher, 1982) and the delay in onset and intensity of induced seizures. Although in the present study, the effect of clonidine on GABA levels did not reach statistical significance, the clonidine treated right hemishpere had a substantially higher GABA level than those in saline or sham groups.

The symptomatic group of animals was not affected by the saline injection, but the morphine injection precipitated stroke symptoms, particularly ipsilateral circling. The level of NE was not affected by the morphine injection but there was a decreased level of DA in the right hemisphere with little effect on the left hemisphere. The decreased level of DA in the right hemisphere seems to correlate with the development of ipsilateral circling behavior (stroke symptom) in the asymptomatic group of animals. Harrison et al., (1979) concluded that

DA changes did not directly contribute to circling behavior because there were no significant changes in DA content after 3.5 hours of ischemia. However, Ahagon et al., (1980) reported the significant participation of dopaminergic neurons in the circling behavior. Circling behavior appears to be related to low DA levels in gerbils. Interestingly, the decreased level of DA was also associated with seizure symptoms, which is consistent with the work of Jobe et al., (1973). They found that brain catecholamine depletion lowered convulsive thresholds in animals. Conversely, drugs which enhance catecholaminergic transmission raise the convulsive threshold. (Meldrum et al., 1975b). Therefore, it may be that ipsilateral circling behavior is a seizure symptom rather than a stroke symptom.

In the neurological deficit group of animals, injection of naloxone did not improve the symptoms, in contrast to the results obtained by Hosobuchi et al., (1982). Similarly, Holaday and D'Amato (1982) were unable to demonstrate a beneficial effect of naloxone.

In Summary, the results of this study indicate that:

1. Naloxone has no beneficial effect in the treatment of acute cerebral ischemia.
2. Morphine may precipitate stroke symptoms.
3. Clonidine at the dose used in this study aborts post ischemia seizure acitivity by preventing increases in NE in the ischemic tissue. This finding may have clinical significance.
4. Decreases in DA and increases in GABA occur during ischemia and the magnitude of the DA and GABA changes are related to the severity of the stroke symptoms.
5. Future experimental studies on neurotransmitter levels in ischemia

should treat animals with seizures as a separate group.

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